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14. ABSTRACT

The overarching objectives of this proposal were to develop novel reagents for the detection and identification of various proteins, antibodies, and antigens, based on the remarkable sensitivity afforded by surface enhanced Raman spectroscopy (SERS). Metal nanoparticles dimers were synthesized that incorporate SERS reporters selectively at the junction between the two metallic spheres, where signal enhancement is at its maximum. The resulting dimeric structures incorporate aptamers (aptatags) or antibodies (antitags) as the recognition elements. Preparation of

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Report Title

Design and Preparation of Nanoparticle Dimers for SERS Detection

ABSTRACT

The overarching objectives of this proposal were to develop novel reagents for the detection and identification of various proteins, antibodies, and antigens, based on the remarkable sensitivity afforded by surface enhanced Raman spectroscopy (SERS). Metal nanoparticles dimers were synthesized that incorporate SERS reporters selectively at the junction between the two metallic spheres, where signal enhancement is at its maximum. The resulting dimeric structures incorporate aptamers (aptatags) or antibodies (antitags) as the recognition elements. Preparation of suitable sensing surfaces will enable target detection down to the level of a few molecules. Furthermore, by correlating the optical signatures of the reporting chromophore with the selectivity of the agent on the antitag or aptatag surface, it should be possible to develop a single sensing surface capable of identifying multiple proteins in complex mixtures.
All of the objectives stated in the original proposal were achieved. Notable highlights include the fine-tuning of chemical steps to produce antitags with femtomolar detection thresholds and the demonstration of multiplexed assays with very high levels of selectivity. A key element for the design of these highly sensitive SERS reporters involve simple to carry out surface passivation chemistry using thiolated polyethyleneglycol prior to the purification steps.

Enter List of papers submitted or published that acknowledge ARO support from the start of the project to the date of this printing. List the papers, including journal references, in the following categories:

(a) Papers published in peer-reviewed journals (N/A for none)

Received	Paper
2012/10/09 1 3	Laura_Fabris, Patrick Z._El-Khoury, Ping_Chu, Desireé D._Whitmore, Guillermo C._Bazan, Eric O._Potma, V. A._Apkarian. High Sensitivity Surface-Enhanced Raman Scattering in Solution Using Engineered Silver Nanosphere Dimers, The Journal of Physical Chemistry C, (08 2011): 15900. doi: 10.1021/jp205055h
2012/10/09 1 2	Laura_Fabris. Bottom-up optimization of SERS hot-spots, Chemical Communications, (07 2012): 9346. doi: 10.1039/c2cc34068b
2011/10/26 1 1	Nekane Guarrotxena, Guillermo C. Bazan. Antibody-functionalized SERS tags with improved sensitivity, Chemical Communications, (07 2011): 0. doi: 10.1039/c1cc12659h

TOTAL: 3

Number of Papers published in peer-reviewed journals:

(b) Papers published in non-peer-reviewed journals (N/A for none)

Received	Paper

TOTAL:

Number of Papers published in non peer-reviewed journals:

(c) Presentations

Insight into the Synthesis, Design and Processing of Narrow Band Gap Organic Semiconducting Polymers for Solar Cell Fabrication, Cavendish Laboratory, Cambridge University, United Kingdom, November 2010.
Insight into the Synthesis, Design and Processing of Narrow Band Gap Organic Semiconducting Polymers for Solar Cell Fabrication, Guanju Institute of Science and Technology, Korea, November 2010.
Insight into the Synthesis, Design and Processing of Narrow Band Gap Organic Semiconducting Polymers for Solar Cell Fabrication, Unam National Institute of Technology, Unam, Korea, November 2010.
Insight into the Synthesis, Design and Processing of Narrow Band Gap Organic Semiconducting Polymers for Solar Cell Fabrication, Materials Research Society – Fall Meeting, Boston, MA, November 2010.
Insight into the Synthesis, Design and Processing of Narrow Band Gap Organic Semiconducting Polymers for Solar Cell Fabrication, Konarka Plastic Power, Lowell, MA, November 2010.
Insight into the Synthesis, Design and Processing of Narrow Band Gap Organic Semiconducting Polymers for Solar Cell Fabrication, Pacifichem 2010 Congress, Honolulu, HI, December 2010.
Design, Synthesis and Processing of Narrow Band Gap Organic Semiconductors for Solar Cell Fabrication, Inter-American Photochemical Conference, Mendoza, Argentina, May 2011.

Number of Presentations: 7.00

Non Peer-Reviewed Conference Proceeding publications (other than abstracts):

Received Paper

TOTAL:

Number of Non Peer-Reviewed Conference Proceeding publications (other than abstracts):

Peer-Reviewed Conference Proceeding publications (other than abstracts):

Received Paper

TOTAL:

Number of Peer-Reviewed Conference Proceeding publications (other than abstracts):

(d) Manuscripts

Received Paper

TOTAL:

Number of Manuscripts:

Books

Received Paper

TOTAL:

Patents Submitted

Patents Awarded

Awards

Graduate Students

NAME	PERCENT_SUPPORTED	Discipline
David P. Stockdale	0.49	
FTE Equivalent:	**0.49**	
Total Number:	**1**	

Names of Post Doctorates

NAME	PERCENT_SUPPORTED
Huaping Li	1.00
Yan Ren	1.00
FTE Equivalent:	**2.00**
Total Number:	**2**

Names of Faculty Supported

NAME	PERCENT_SUPPORTED	National Academy Member
Dr. Guillermo Bazan	0.11	Yes
FTE Equivalent:	**0.11**	
Total Number:	**1**	

Names of Under Graduate students supported

NAME	PERCENT_SUPPORTED
FTE Equivalent:	
Total Number:	

Student Metrics

This section only applies to graduating undergraduates supported by this agreement in this reporting period

The number of undergraduates funded by this agreement who graduated during this period:	0.00
The number of undergraduates funded by this agreement who graduated during this period with a degree in science, mathematics, engineering, or technology fields:.....	0.00
The number of undergraduates funded by your agreement who graduated during this period and will continue to pursue a graduate or Ph.D. degree in science, mathematics, engineering, or technology fields:.....	0.00
Number of graduating undergraduates who achieved a 3.5 GPA to 4.0 (4.0 max scale):	0.00
Number of graduating undergraduates funded by a DoD funded Center of Excellence grant for Education, Research and Engineering:	0.00
The number of undergraduates funded by your agreement who graduated during this period and intend to work for the Department of Defense	0.00
The number of undergraduates funded by your agreement who graduated during this period and will receive scholarships or fellowships for further studies in science, mathematics, engineering or technology fields:	0.00

Names of Personnel receiving masters degrees

NAME
Total Number:

Names of personnel receiving PHDs

NAME
Total Number:

Names of other research staff

NAME	PERCENT_SUPPORTED
Xiaofen Chen	0.71
Dr. Nekane Guarrotxena	0.81
Dr. Huiping Wang	0.31
FTE Equivalent:	**1.83**
Total Number:	**3**

Sub Contractors (DD882)

Inventions (DD882)

Scientific Progress

Technology Transfer

Final Technical Report

Design and Preparation of Nanoparticle Dimers for SERS Detection

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University of California, Santa Barbara, CA 93103

Abstract

The overarching objectives of this proposal were to develop novel reagents for the detection and identification of various proteins, antibodies, and antigens, based on the remarkable sensitivity afforded by surface enhanced Raman spectroscopy (SERS). Metal nanoparticles dimers were synthesized that incorporate SERS reporters selectively at the junction between the two metallic spheres, where signal enhancement is at its maximum. The resulting dimeric structures incorporate aptamers (aptatags) or antibodies (antitags) as the recognition elements. Preparation of suitable sensing surfaces will enable target detection down to the level of a few molecules. Furthermore, by correlating the optical signatures of the reporting chromophore with the selectivity of the agent on the antitag or aptatag surface, it should be possible to develop a single sensing surface capable of identifying multiple proteins in complex mixtures.

All of the objectives stated in the original proposal were achieved. Notable highlights include the fine-tuning of chemical steps to produce antitags with femtomolar detection thresholds and the demonstration of multiplexed assays with very high levels of selectivity. A key element for the design of these highly sensitive SERS reporters involve simple to carry out surface passivation chemistry using thiolated polyethyleneglycol prior to the purification steps.

Scientific Progress and Accomplishments

Innovative biosensory approaches based on the properties of colloidal nanoparticle (NP) assemblies have emerged with the potential to reach single-molecule detection thresholds.[1,2] Methodologies based on surface enhanced Raman spectroscopy (SERS) benefit from the electromagnetic field enhancement due to the collective excitation of electrons in metallic nanostructures.[3,4] This enhancement can occur within inter-nanoparticle gaps,[4b,5] and several strategies exist for achieving the requisite assemblies.[6-8] Another useful distinguishing feature of SERS-based detection strategies arises from the narrow spectral bandwidths.[8c,9]

We reported in previous progress reports multi-NP structures termed ¨antitags¨ that incorporate SERS reporters within suitable intersticial sites and antigen-specific recognition elements for protein detection in heterogeneous assays.[10] Antitags, as shown in Figure 1a, consist of silver NPs (typically ~35-nm) held together by a dithiolated linker and an antibody-functionalized polyethylene glycol (PEG) coating. This thin layer is bifunctional by design, and contains thiol groups for binding to the NP surface and carboxylic functionalities for coupling with suitable antibody probes.

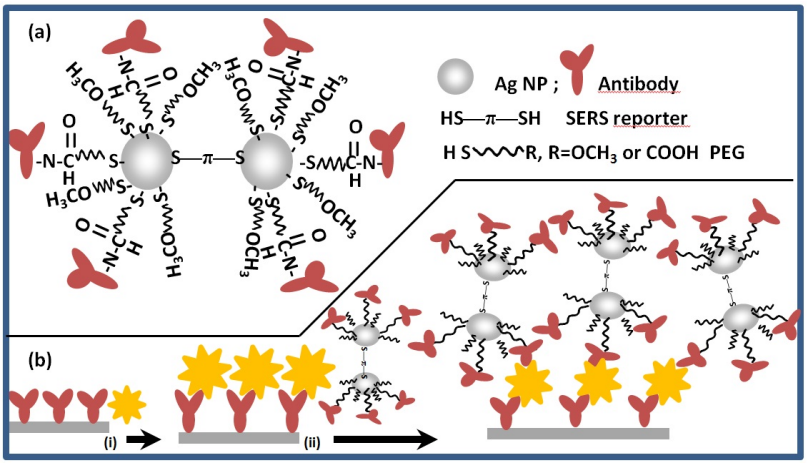

Figure 1. (a) Schematic illustration of the antibody functionalized antitags. (b) SERS-based ELISA-analog detection. Human α-thrombin (Thr) antigen (yellow) is exposed to an epoxy functionalized glass substrate modified with a covalently immobilized layer of human α-thrombin mouse monoclonal capturing antibodies (pink). The surface-bound Thrs subsequently couple with antitags via specific protein/antibody interactions. Drawing is not to scale.

Antitags have enabled sensitive SERS analogs of the enzyme-linked immunosorbent assay (ELISA). Picomolar detection limits have been reported.[10] The overall action of the bioassay is depicted in Figure 1b and is based on the binding specificity of antibody/antigen pairs and the amplification properties of SERS. The procedure is described below in more detail. Figure 1a shows an idealized NP dimer, but it should be noted that higher order aggregates can also function as long as they do not non-specifically bind to the assay substrate. It is also worth pointing out that the ability to utilize different SERS labels paves the way for multiplexed detection on a single sensing surface, a feature currently not possible with the colorimetric or fluorometric ELISA counterparts.[10] During this previous funding period, and described in more detail below, we have shown important improvements in antitag sensitivity by properly managing surface properties so that aggregation is minimized.

Antitags have been previously prepared by the sequence of steps highlighted in blue as shown in Figure 2.[10] Biphenyl-4,4'-dithiol (DBDT) was first used to link the NPs together, step (i), and to serve as the SERS reporter. After centrifugation and isolation, step (ii), NP dimers and higher aggregates were stabilized by the addition of thiolated carboxylic-polyethylene glycol (HS-PEG-COOH), step (iii). This step minimizes protein-induced NP aggregation in subsequent treatments[11] and incorporates carboxylic functionalities that serve to anchor antibodies, as in step (iv), via carbodiimide-mediated amidation.[12] An additional methoxy-terminated PEG layer, step (v), is included to further stabilize toward against ligand exchange and aggregation.[4b,13] However, examination of different batches revealed that the conditions used for centrifugation could influence the final distribution of NP aggregates, i.e. the ratio of monomer, dimer, trimer, and higher order species.

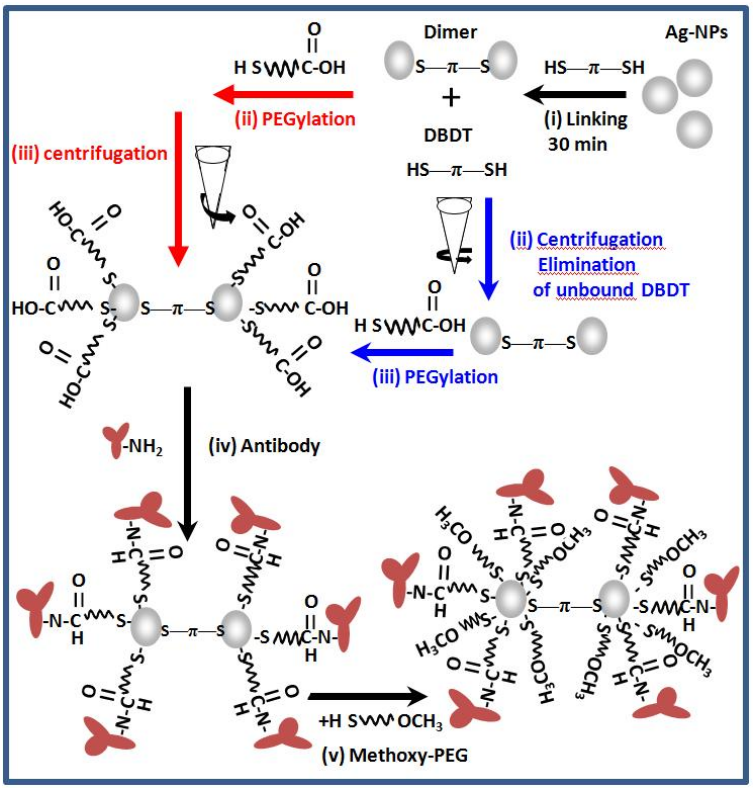

Figure 2. Schematic of the preparation of SERS active antitags via new (red) and previous[6] (blue) procedure.

To address the batch to batch variability described above, a modified SERS antitag synthetic procedure was examined. The new process is highlighted in red in Figure 2. Essentially, HS-PEG-COOH is added to the reaction mixture prior to centrifugation with the idea that surface passivation via incorporation polar functionalities and steric bulk would prevent undesired aggregation.[14] Subsequent procedures after the colored sequences in Figure 2 were kept similar.

Figure 3 shows transmission electron microscopy (TEM) images collected from the NP distributions obtained via the two procedures in Figure 2. These micrographs show that the preventive coating HS-PEG-COOH prior to centrifugation, i.e. the red sequence of steps in Figure 2, yields a distribution of aggregates biased toward smaller number of incorporated NPs.

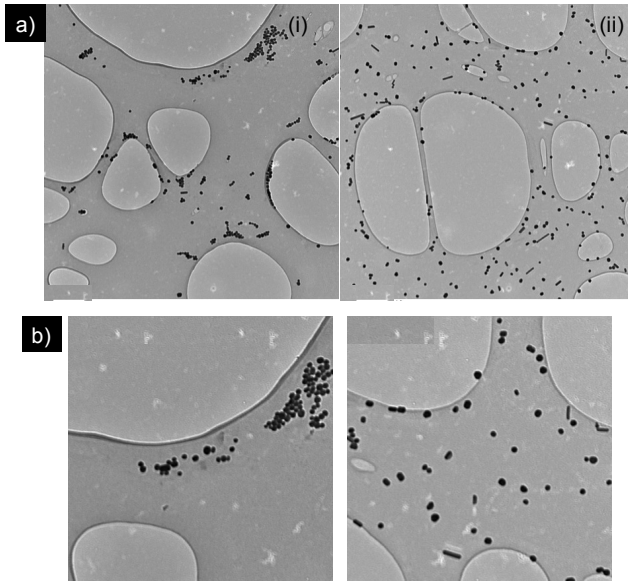

Figure 3. a) TEM micrographs of NPs obtained via centrifugation/ passivation (i) and passivation/centrifugation (ii). b) Close up images of PEG coated NPs by (i) and (ii) process.

We utilized the sandwich assay shown in Figure 1b to detect the presence of human a-thrombin (Thr) antigen and thus demonstrate the sensing function of the new antitags. In this approach, the protein is first captured by human a-thrombin mouse monoclonal antibodies immobilized on an epoxy-functionalized glass substrate. Antitags with the reporting antibody specifically bind to the surface-bound Thr antigens. After washing away unbound antitags, the Raman signals can be correlated to surface bound Thr. Concentrations of 10^2, 10^3, 10^4, 10^5, 10^6 fM of Thr were examined using the diagnostic fingerprint of DBDT-Raman reporter (1589 cm^{-1}), as obtained with a 633 nm laser source and 1 s exposure time. Figure 4 displays representative spectra for the thrombin detection platform using the improved antitags.

Inspection of Figure 4 shows that the intensity of the peak increases with increasing Thr concentration. Figure 5 provides a more quantitative measure by plotting the ratio of the 1589 cm^{-1} peak intensity in the presence and absence of Thr as a function of concentrations. Each data point represents the average of four measurements from 15mm x 15mm surfaces on the same substrate. A control sample was prepared under the same assay conditions with the absence of Thr (red curve in Figure 4). Differentiation between control samples and thrombin coated surfaces exhibits a detection range from picomolar to femtomolar levels. The error bars represent the standard deviation as obtained from three different substrates. This dose-response curve was fitted linearly with a resulting R square value of 0.9962. The 100 fM limit of detection (LOD) is based on a 3:1 threshold ratio with respect to the control measurement.

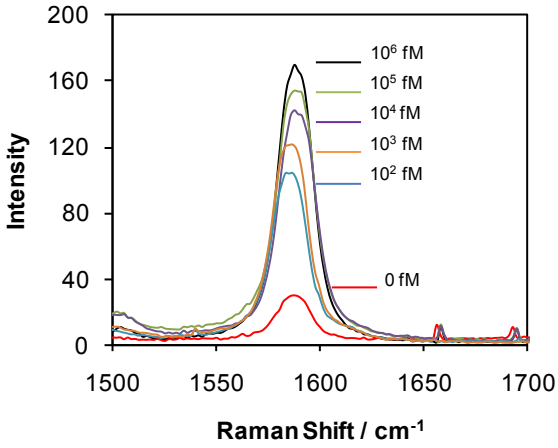

Figure 4. Representative results from the SERS-based immunoassay detection of thrombin collected at different protein concentrations: 10^2 fM, 10^3 fM, 10^4 fM, 10^5 fM and 10^6 fM. The control sample (0 fM) is obtained in the absence of thrombin.

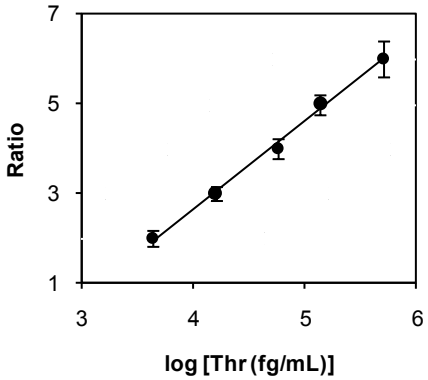

Figure 5. Dose-response curve from the antibody-antigen binding affinity using a range of thrombin concentrations (100fM-1 nM). The best-fit line is as follows: y=1.9688 x – 5.2298 (R^2=0.9962). The error bars represent the standard deviation. Ratio is the coefficient between the 1589 cm^{-1} peak intensity in the presence and absence of the protein.

The results shown in Figure 5 demonstrate that using antitags prepared via the new procedure offers more than a 100- and 1000-fold improvement in the detection limit over that achievable with commercially available ELISA kits (~10 pM)[15] and the performance of antitags prepared via the previous synthetic route (100 pM), respectively.[10] In addition, this assay provides higher sensitivity than the previous literature report of 0.5 pM.[2d,16]

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